

Claims

5        1. A method for providing a nucleic acid from a sample without use of a cell lysing reagent, comprising the steps of:

10        A) at a pH of less than 7, contacting a sample suspected of containing a nucleic acid with a water-soluble, weakly basic polymer comprised of recurring units derived by addition polymerization of:

15        1) from about 15 to 100 weight percent of a water-soluble, weakly basic ethylenically unsaturated polymerizable monomer having at least one group which can be protonated at acidic pH and which is selected from the group consisting of aminoalkyl, imidazolyl, isoxazolyl, pyridyl, piperidyl, piperazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, pyridazinyl, pyrimidyl, pyrazinyl, quinolinyl and quinazolinyl,

20        2) from 0 to about 35 weight percent of a nonionic, hydrophilic ethylenically unsaturated polymerizable monomer, and

25        3) from 0 to about 85 weight percent of a nonionic, hydrophobic ethylenically unsaturated polymerizable monomer in an amount sufficient to form a water-insoluble precipitate of said weakly basic polymer with all nucleic acids present in said lysate,

B) separating said water-insoluble precipitate from said sample, and

C) contacting said precipitate with a base to raise the solution pH to greater than 7, and thereby releasing said nucleic acids from said weakly basic polymer,

5           said weakly basic polymer comprising recurring units derived by addition polymerization of one or more ethylenically unsaturated polymerizable monomers having an amine group which can be protonated at acidic pH.

2. The method of claim 1 further comprising the step:

10           D) adjusting the pH of said solution containing said released nucleic acids to from about 6 to about 9..

15           3. The method of claim 1 wherein said base is sodium hydroxide, potassium hydroxide, ammonium hydroxide, lithium hydroxide, sodium carbonate, sodium bicarbonate, a tertiary amine or tris(hydroxymethyl)-aminomethane.

20           4. The method of claim 1 wherein said weakly basic polymer is used in step A) in an amount of from about 0.01 to about 0.5 weight %.

5. The method of claim 1 wherein a weak base is used in step C), accompanied by heating said water-insoluble precipitate at from about 50° to about 125° C.

25           6. The method of claim 1 wherein a strong base is used in step C) without heating said water-insoluble precipitate.

7. A method for the amplification and detection of a target nucleic acid without the use of a cell lysing reagent, comprising:

I) providing a sample suspected of containing a target nucleic acid,

II) subjecting said sample containing the target nucleic acid to the steps of:

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A) at a pH of less than 7, contacting said target nucleic acid with a water-soluble, weakly basic polymer comprised of recurring units derived by addition polymerization of:

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1) from about 15 to 100 weight percent of a water-soluble, weakly basic ethylenically unsaturated polymerizable monomer having at least one group which can be protonated at acidic pH and which is selected from the group consisting of aminoalkyl, imidazolyl, isoxazolyl, pyridyl, piperidyl, piperazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, pyridazinyl, pyrimidyl, pyrazinyl, quinolinyl and quinazolinyl,

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2) from 0 to about 35 weight percent of a nonionic, hydrophilic ethylenically unsaturated polymerizable monomer, and

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3) from 0 to about 85 weight percent of a nonionic, hydrophobic ethylenically unsaturated polymerizable monomer in an amount sufficient to form a water-insoluble precipitate of said weakly basic polymer with all nucleic acids present in said sample, including said target nucleic acid,

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B) separating said water-insoluble precipitate from said sample, and

C) contacting said precipitate with a base to raise the solution pH to greater than 7, and thereby releasing said nucleic acids, including said target nucleic acid, from said weakly basic polymer,

5           said weakly basic polymer comprising recurring units derived by addition polymerization of one or more ethylenically unsaturated polymerizable monomers having an amine group which can be protonated at acidic pH,

10          III) without further adjustment of pH, amplifying said released target nucleic acid, and

IV) detecting said amplified target nucleic acid.

15          8. The method of claim 7 wherein said weakly basic polymer is water-insoluble at basic pH, and said method further comprises the step of removing said water-insoluble polymer after release of said target nucleic acid therefrom and prior to amplification thereof.

20          9. The method of claim 7 wherein the target nucleic acid is a K-ras sequence.

20          10. A kit for detection of K-ras mutation in a biological sample, said kit comprising a diagnostic K-ras primer selected from the group consisting of:  
1>       (a) TGAATATAAA CTTGTGGTAC CTGGAGC T (5K15S) <SEQ ID: NO  
2>       (b) ATATAAAACTT GTGGTAGTTC CAGCTGGT (5K37) <SEQ ID: NO  
3>       (c) GAATTAGCTG TATCGTCAAG GCACTC (3K42)   <SEQ ID: NO  
4>       (d) TCAGCAAAGA CAAGACAGGT A (5BK5)   <SEQ ID: NO 4>,  
5>       (e) TATAGATGGT GAAACCTGTT TGTTGG (5N12A)   <SEQ ID: NO  
6>       (f) CTTGCTATTA TTGATGGCAA CCACACAGA (3N13A)   <SEQ ID:  
NO 6

(g) any combination of the foregoing.

11. The oligonucleotide TGAATATAAAA CTTGTGGTAC CTGGAGC T <SEQ ID: NO 1>.
12. The oligonucleotide ATATAAACTT GTGGTAGTTC CAGCTGGT <SEQ ID: NO 2>.
13. The oligonucleotide GAATTAGCTG TATCGTCAAG GCACTC <SEQ ID: NO 3>.
14. The oligonucleotide TCAGCAAAGA CAAGACAGGT A <SEQ ID: NO 4>.
15. The oligonucleotide TATAGATGGT GAAACCTGTT TGTTGG <SEQ ID: NO 5>.
16. The oligonucleotide CTTGCTATT A TTGATGGCAA CCACACAGA <SEQ ID: NO 6>.
17. A K-ras diagnostic primer comprising the oligonucleotide TGAATATAAAA CTTGTGGTAC CTGGAGC T <SEQ ID: NO 1>.
18. A K-ras diagnostic primer comprising the oligonucleotide ATATAAACTT GTGGTAGTTC CAGCTGGT <SEQ ID: NO 2>.
19. A K-ras diagnostic primer comprising the oligonucleotide GAATTAGCTG TATCGTCAAG GCACTC <SEQ ID: NO 3>.
20. A K-ras diagnostic primer comprising the oligonucleotide TCAGCAAAGA CAAGACAGGT A <SEQ ID: NO 4>.
21. A K-ras diagnostic primer comprising the oligonucleotide TATAGATGGT GAAACCTGTT TGTTGG <SEQ ID: NO 5>.
22. A K-ras diagnostic primer comprising the oligonucleotide CTTGCTATT A TTGATGGCAA CCACACAGA <SEQ ID: NO 6>.

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